

## RESEARCH ARTICLE

## Progranulin mutations in clinical and neuropathological Alzheimer's disease

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## Funding information

National Human Genome Research Institute; Large Scale Sequencing and Analysis Centers (LSAC); the National Heart, Lung, and Blood Institute (NHLBI); National Institute of Health (NIH); National Institute on

## Abstract

**Introduction:** Progranulin (*GRN*) mutations occur in frontotemporal lobar degeneration (FTLD) and in Alzheimer's disease (AD), often with TDP-43 pathology.

**Methods:** We determined the frequency of rs5848 and rare, pathogenic *GRN* mutations in two autopsy and one family cohort. We compared Braak stage,  $\beta$ -amyloid load, hyperphosphorylated tau (PHFtau) tangle density and TDP-43 pathology in *GRN* carriers and non-carriers.

**Results:** Pathogenic *GRN* mutations were more frequent in all cohorts compared to the Genome Aggregation Database (gnomAD), but there was no evidence for association

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Aging, Grant/Award Numbers: P30AG10161, R01AG15819, R01AG17917, R01AG42210, U01AG46152, U01AG61356, R56AG063908, R01AG067501, RF1AG015473, U01AG032984, UM1HG008901, R01AG033193, U01AG006781, RF1AG054023, RF1AG015473, R01AG048927, RF1AG057519, R03AG054936, AG033193

with AD. Pathogenic *GRN* carriers had significantly higher PHFtau tangle density adjusting for age, sex and *APOE*  $\epsilon$ 4 genotype. AD patients with rs5848 had higher frequencies of hippocampal sclerosis and TDP-43 deposits. Twenty-two rare, pathogenic *GRN* variants were observed in the family cohort.

**Discussion:** *GRN* mutations in clinical and neuropathological AD increase the burden of tau-related brain pathology but show no specific association with  $\beta$ -amyloid load or AD.

#### KEYWORDS

Alzheimer's disease, neuropathology, progranulin, TDP43

## 1 | INTRODUCTION

Alzheimer's Disease (AD) is the primary cause of dementia among older people with a strong genetic predisposition<sup>1</sup> (60% to 80% heritability), a prevalence of 30% at age 70 years and an annual incidence rate of 6% to 8% by age 85 years.<sup>2</sup> Extra-cellular accumulation and deposition of  $\beta$ -amyloid ( $A\beta$ ) in the brain is thought to be an early event. Although phosphorylated tau is thought to have a role in the cause of AD its role in pathogenesis is uncertain. Understanding the biological mechanisms of AD could reveal insights about its etiology, and aid in the development of novel treatments and pre-symptomatic diagnosis.<sup>3,4</sup>

Progranulin, a microglial protein encoded by the *GRN* gene, is neurotrophic and anti-inflammatory, and there is increased expression by microglia in conditions of pathology.<sup>5</sup> *GRN* mutations are consistently associated with frontotemporal lobar degeneration (FTLD)<sup>6</sup> but recent genetic and epidemiological studies suggest that *GRN* variants may also be observed in AD. *GRN* depletion heightens  $A\beta$  and tau deposition in mice, and its expression rises in microglia surrounding plaques.<sup>7-9</sup>

Progranulin levels are increased in the cerebrospinal fluid (CSF) of patients with both an autosomal-dominant early onset AD and sporadic late-onset AD.<sup>10</sup> *GRN* mutations in patients with clinical AD have been previously reported in large families in the National Institute on Aging family-based study,<sup>11</sup> among large, multiply affected families of Caribbean Hispanic ancestry<sup>12</sup> and in patients from a large exome-sequencing study.<sup>13</sup> A family clinically diagnosed with AD and also carrying a *GRN* mutation (c.154delA) had FTLD with ubiquitin-positive, tau-negative, and lentiform neuronal intranuclear inclusions (FTLD-U NII) with neuronal loss and gliosis, affecting the frontal and temporal lobes, and TDP-43 inclusions.<sup>14</sup> Only one of the six family members had mixed pathology meeting NIA-Reagan criteria<sup>15</sup> of high likelihood and coexisting FTLD-U N11 with TDP-43 inclusions. *GRN* mutations were also observed in a patient with postmortem evidence of AD: NIA-Reagan criteria of high likelihood<sup>15</sup> and coexisting FTLD-U N11 with TDP-43 inclusions.<sup>16</sup>

Here we investigated the frequency of pathogenic *GRN* mutations in large unrelated AD cohorts and in families among patients with either clinical or postmortem AD. In clinical AD, we compared the frequency of behavioral and other symptoms (such as learning disabilities) consistent with a FTLD presentation. In autopsied-confirmed AD, we evalu-

ated the presence of co-pathologies including tauopathies and TDP-43 presentation.

## 2 | METHODS

### 2.1 | ROSMAP cohort

#### 2.1.1 | Cohorts and whole genome sequencing (WGS)

Whole genome sequencing (WGS) data from 1161 autopsied brain tissues were accessed from the ROSMAP cohort which is comprised of two prospective studies of aging—The Religious Orders Study (ROS) and the Memory and Aging Project (MAP). The detailed description of the study design and data collection scheme are described elsewhere.<sup>17-19</sup> All individuals have longitudinal clinical assessments of AD based on the NINCDS-ADRDA criteria<sup>20,21</sup> and neuropathological diagnosis based on the NIA-Reagan criteria.<sup>15</sup> We defined neuropathological diagnosis of AD as having a NIA-Reagan score of 1 or 2 (high or intermediate likelihood of disease). Both studies were approved by an Institutional Review Board, and all participants signed an informed consent, Anatomic Gift Act, and a repository consent to all their data to be shared. WGS was performed at the New York Genome Center using DNA extracted from brain tissue ( $n = 806$ ), whole blood ( $n = 389$ ), or lymphocytes transformed with EBV virus ( $n = 5$ ). Details of the sequencing technology and bioinformatics pipeline for data processing, read alignment, and variant calling have been described.<sup>22</sup>

#### 2.1.2 | Correlation of *GRN* mutations with neuropathological phenotypes

We first evaluated the frequency of rare putatively pathogenic *GRN* variants in the ROSMAP autopsy cohort. Pathogenicity was defined as coding mutations that have a Combined Annotation Dependent Depletion (CADD) greater than 20 or mutations that affect splicing. Joint frequency of *GRN* mutations was defined as the sum of minor allele frequencies (MAF) of pathogenic mutations. We then correlated the *GRN* mutation dosage (number of mutations carried by each individual)

with neuropathological traits. Neuropathological traits included (1) global pathology defined as a global measure of pathology based on the scaled scores of five brain regions where the scaled variable is the original count divided by the standard deviation; (2) Braak Stage<sup>23</sup>; (3) diffuse plaque burden; (4) neuritic plaque burden; (5) hyperphosphorylated tau (PHFtau) tangle density across eight brain regions; (6) area occupied by A $\beta$  across eight brain regions; (7) hippocampal sclerosis (present/absent); (8) TDP-43 inclusions (present/absent); (9) synaptic measure across three cortical regions (hippocampus, midfrontal cortex, and inferior temporal); and (10) presence of Lewy bodies. Three stages of TDP-43 pathology were measured (stage 1, localized to amygdala; stage 2, extension to hippocampus, and/or entorhinal cortex; stage 3, extension to the neocortex), and the severity of the TDP-43 cytoplasmic inclusions in neurons and glia were rated on a six-point scale.<sup>24</sup>

Correlations were computed as follows: (1) unadjusted; (2) adjusted for age at death and sex; (3) adjusted for age at death, sex, and pathological AD diagnosis. Pathological AD was derived using the NIA-Reagan diagnosis of Alzheimer's disease.<sup>25</sup>

## 2.2 | The National Alzheimer's Coordinating Center (NACC)

### 2.2.1 | Cohort and WGS

The NACC coordinated collection of phenotype data from the 29 National Institute on Aging (NIA) Alzheimer's Disease Centers (ADCs), stored and shared all data, coordinated the adjudication of AD cases and controls, and collection of samples. For autopsy samples, clinical and neuropathologic information were recorded in either the minimal dataset (MDS) or the more extensive uniform data set (UDS) (after 2006), and neuropathologic information was recorded in the Neuropathology Data Set. Details of the cohort have been reported.<sup>26,27</sup> Clinical diagnosis of AD was based on the NINCDS-ADRDA criteria<sup>20,21</sup> and neuropathological AD was defined as a score of 1 or 2 (high or intermediate likelihood) on the NIA-AA Alzheimer's disease neuropathologic change (ADNC) scale.<sup>15</sup>

Whole exome sequencing (WES) data for the NACC autopsied individuals were generated as a part of the AD Sequencing Project and were accessed from The National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site.<sup>28</sup> The study design and details of the WES experiment and variant calling are described elsewhere.<sup>29,30</sup>

### 2.2.2 | Correlation of GRN mutations with neuropathological measures

GRN mutation dosage defined as the sum of non-reference alleles in pathogenic mutations was correlated with presence of FTLN with tau (FTLN-tau) pathologies such as argyrophilic grains, tau intracytoplasmic inclusions, TDP-43 inclusions, neurofibrillary tangles, or pre-tangles (see Mann and Snowden<sup>31</sup>) from the NACC MDS and UDS. The

## RESEARCH IN CONTEXT

- 1. Systematic review:** Common variants and rare progranulin (*GRN*) mutations, typically associated with FTLN, have been identified in genome wide arrays and genome wide sequencing of Alzheimer's disease (AD). We sought to determine whether *GRN* variants were specifically associated with AD and establish their impact on the disease phenotype.
- 2. Interpretation:** We found the frequency of *GRN* mutations among patients with AD ranged from 0.5% in unrelated individuals to 5% in families, but there was no specific association with clinical or pathological AD. Between carriers and non-carriers there were no statistical differences in behavioral manifestations. Compared with non-carriers at autopsy, patients with AD and *GRN* mutations had advanced Braak stages, increased tangle density, TDP-43 pathology, and evidence of other tauopathies.
- 3. Future directions:** *GRN* mutations are not associated with an increased risk of AD, but when present in neuropathological AD alter the phenotype by increasing the burden of tau-related brain pathology.

proportion of individuals with clinical AD and with FTLN-tau pathology were compared between *GRN* mutation carriers and non-carriers.

## 2.3 | Estudio Familiar de Influencia Genética en Alzheimer's (EFIGA)

### 2.3.1 | Cohort and WGS

WGS data from 307 families in the Estudio Familiar de Influencia Genética en Alzheimer's (EFIGA) cohort were accessed. Study design, adjudication, and clinical assessment of AD in this cohort were previously described.<sup>32</sup> Participants were followed up every 2 years with a neuropsychological test battery,<sup>33</sup> a structured medical and neurological examination, and an assessment of depression.<sup>34,35</sup> The Clinical Dementia Rating Scale<sup>36,37</sup> was administered and functional status was assessed, and the clinical diagnosis of AD was based on the NINCDS-ADRDA criteria.<sup>20,21</sup> Seventy-seven families in EFIGA underwent sequencing as a part of the Alzheimer's Disease Sequencing Project (ADSP) discovery and extension phases.<sup>38</sup>

WGS on 1886 individuals from 264 families was also performed at the New York Genome Center (NYGC) using one microgram of DNA, an Illumina PCR-free library protocol, and sequencing on the Illumina HiSeq platform.

We harmonized the WGS the EFIGA families (n = 307), and jointly called variants to create a uniform analysis set. Genomes were sequenced to a mean coverage of 30x. Sequence data analysis was performed using the NYGC automated analysis pipeline which matches the centers for common disease genomics (CCDG) and

Trans-Omics for Precision Medicine recommended best practices.<sup>39</sup> Briefly, sequencing reads were aligned to the human reference, hs38DH, using BWA-MEM v0.7.15. Variant calling was performed using the Genome Analysis Toolkit best-practices. Variant filtration was performed using Variant Quality Score Recalibration (at tranche 99.6%) which identified annotation profiles of variants that were likely to be real and assigns a score to each variant.

### 2.3.2 | Correlation of GRN variants with clinical assessment of FTLD-like symptoms

Behavioral traits associated with FTLD had been collected in a subgroup of the EFIGA cohort and was compared in those with clinical AD with and without pathogenic GRN mutations. Presence of FTLD-like behavioral symptoms were assessed on a ten-point Middelheim Frontality Score (MFS).<sup>40</sup>

## 2.4 | Statistical analysis

Partial correlations (cor) adjusting for covariates were computed using the ppcor R package<sup>41</sup> and results were assessed for significance at  $P \leq 0.05$ .

## 3 | RESULTS

### 3.1 | Frequency of GRN mutations

We annotated mutations from the AD and FTD Mutation Database (<https://uantwerpen.vib.be/>) to assess the CADD scores of putatively deleterious variants in GRN (Figure S1 in the Supporting Information). Of the 171 mutations in the AD and FTD databases, 78 (45%) were classified as "pathogenic" and 45 (26%) were considered "unclear," with average CADD scores of 28.4 ( $\pm 7.6$ ) and 19.3 ( $\pm 9.73$ ) respectively. Thus, we used CADD  $\geq 20$  to define pathogenic GRN loss of function, non-synonymous, and splice variants mutations.

Table 1 shows the frequency of pathogenic GRN mutations in each dataset. Only summary level data were available from the

**TABLE 1** Frequency of pathogenic GRN mutations with CADD score  $\geq 20$  in the different cohorts

Dataset	Number of variants	Frequency in affected	Frequency in unaffected
gnomAD exomes (all populations)	127	NA	0.0075
EFIGA families	22	0.0512	0.0473
ROSMAP	8	0.014	0.0082
NACC	30	0.0052	0.0052

Abbreviations: CADD, Combined Annotation Dependent Depletion; gnomAD, Genome Aggregation Database; EFIGA, Estudio Familiar de Influenza Genética en Alzheimer's; ROSMAP, Religious Orders Study and Memory and Aging Project; NACC, National Alzheimer's Coordinating Center.

Genome Aggregation Database (gnomAD) and the total frequency of pathogenic variants was assessed as the sum of frequencies of individual variants (assuming that each variant was observed once in an individual). The population frequency of pathogenic GRN variants in gnomAD was 0.75%. In the EFIGA family cohort, there was a significant enrichment of pathogenic GRN mutations, although no significant differences were observed between clinical AD and unaffected family members. In the ROSMAP study, the frequency of GRN mutations in post-mortem AD cases was observed at 1.4% for the affected and 0.8% for the controls cohort.

### 3.2 | Association of GRN with neuropathological traits in ROSMAP

We observed eight pathogenic GRN mutations at a MAF = 1.4% in autopsy confirmed cases and 0.8% in controls. We assessed the correlation of GRN carrier status with neuropathological, behavioral, and cognition-related traits (Table 2, Figures S2-S3 and Table S3). GRN mutations in both cases and controls was accompanied by an advanced Braak Stage (cor = 0.06,  $P = 0.04$ ) and higher PHFtau tangle density (cor = 0.08,  $P = 0.008$ ). Adjusting for age, sex, and AD diagnosis, correlation with PHFtau tangle density was statistically significant (cor = 0.065,  $P = 0.02$ ). The association was significant after adjustment for APOE  $\epsilon 4$  (cor = 0.06,  $P = 0.048$ ). Upon further analysis of GRN and APOE  $\epsilon 4$  (Figures S2-S3), we found higher tangle density in AD patients and healthy individuals who carried both a GRN mutation and APOE  $\epsilon 4$  alleles. This observation was particularly strong in tangle density measured in the entorhinal cortex and the hippocampus. However, this pattern was observed in only five AD patients and two unaffected individuals. There was no association of GRN variants with other neuropathological traits.

Of the 20 individuals in ROSMAP with a neuropathological diagnosis of AD and carrying a GRN mutation, 9 (45%) showed TDP-43 inclusions that were either stage 2 (extension to hippocampus and/or entorhinal cortex) or stage 3 (extension to the neocortex). Moderate to severe TDP-43 pathology was slightly higher in GRN mutation carriers with a confirmed neuropathological diagnosis of AD (45% vs 39.5%). In addition, one patient with confirmed AD and a second individual without dementia but a carrier of a GRN variant had neuropathological characteristics of hippocampal sclerosis.

### 3.3 | Single nucleotide polymorphism (SNP) rs5848 in ROSMAP cohort

The rs5848 single nucleotide polymorphism (SNP), located in the 3'-untranslated region of GRN, predicted to be a binding site for the microRNA miR-659, is the most frequent GRN variant associated with frontotemporal dementia.<sup>42</sup> Several small independent and meta-analysis studies from several populations have reported association of the T allele of rs5848 with risk for clinical AD.<sup>43</sup> Recently, a large meta-analysis of genome-wide association studies (39,106 clinically

**TABLE 2** GRN carrier status is correlated positively with tangle load and Braak stage

	Unadjusted model		Adjusted for age and sex		Adjusted for age, sex and AD status		Adjusted for age, sex and APOE ε4 dosage	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Global pathology <sup>a</sup>	0.04	1.79E-01	0.03	3.32E-01	0.01	6.93E-01	0.02	5.21E-01
Braak Stage	0.06	4.10E-02	0.05	9.04E-02	0.04	1.72E-01	0.04	1.36E-01
Diffuse plaque burden	0.02	5.43E-01	0.02	5.64E-01	0.00	9.29E-01	0.01	7.41E-01
Neuritic plaque burden	0.03	3.27E-01	0.02	5.85E-01	-0.01	8.63E-01	0.01	8.27E-01
square-root of tangle density across eight brain regions	0.08	8.83E-03	0.07	2.68E-02	0.06	4.93E-02	0.06	4.88E-02
square-root of the overall amyloid levels	0.03	3.61E-01	0.02	4.88E-01	0.00	9.86E-01	0.01	6.96E-01
Hippocampal sclerosis (present/absent)	-0.01	7.88E-01	-0.01	7.31E-01	-0.01	6.96E-01	-0.01	6.61E-01
TPD-43 pathology (present/absent)	0.03	4.27E-01	0.02	4.37E-01	0.02	4.93E-01	0.02	4.82E-01
Presence of Lewy bodies	0.00	9.60E-01	0.00	9.78E-01	0.00	9.72E-01	0.00	9.98E-01
Synaptic measure <sup>b</sup>	0.05	2.91E-01	0.06	2.20E-01	0.06	2.05E-01	0.05	2.47E-01

<sup>a</sup>Global pathology defined as global measure of pathology based on the scaled scores of pathology in five brain regions, where the scaled variable is the original count divided by the standard deviation.

<sup>b</sup>Synaptic measure across three cortical (hippocampus, midfrontal cortex, and inferior temporal).

**TABLE 3** Correlation of pathological measures with rs5848 in the ROSMAP cohort

	Unadjusted model		Adjusted for age and sex		Adjusted for age, sex, and AD status		Adjusted for age, sex, and APOE ε4 dosage	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Global pathology <sup>a</sup>	-0.02	4.45E-01	-0.02	4.84E-01	-0.03	3.76E-01	-0.03	3.47E-01
Braak Stage	-0.03	3.40E-01	-0.02	4.91E-01	-0.02	4.14E-01	-0.02	4.86E-01
Diffuse plaque burden	-0.01	6.26E-01	-0.01	6.73E-01	-0.01	6.56E-01	-0.02	5.54E-01
Neuritic plaque burden	-0.01	6.86E-01	-0.01	7.06E-01	-0.01	6.57E-01	-0.02	5.57E-01
Square-root of tangle density across eight brain regions	-0.02	5.22E-01	-0.01	6.64E-01	-0.02	5.87E-01	-0.02	5.34E-01
Square-root of the overall amyloid levels	-0.02	5.78E-01	-0.01	7.51E-01	-0.01	6.76E-01	-0.02	5.51E-01
Hippocampal sclerosis (present/absent),	0.08	4.88E-03	0.09	3.08E-03	0.09	3.09E-03	0.08	4.55E-03
TPD-43 pathology (present/absent)	0.08	1.84E-02	0.08	1.00E-02	0.08	9.52E-03	0.08	1.65E-02
Presence of Lewy bodies	0.00	8.82E-01	0.00	9.00E-01	0.00	9.08E-01	0.00	9.62E-01
Synaptic measure <sup>b</sup>	0.00	9.68E-01	0.00	9.76E-01	0.00	9.37E-01	0.00	9.88E-01

Abbreviation: ROSMAP, Religious Orders Study and Memory and Aging Project.

<sup>a</sup>Global pathology defined as global measure of pathology based on the scaled scores of pathology in five brain regions, where the scaled variable is the original count divided by the standard deviation.

<sup>b</sup>Synaptic measure across three cortical (hippocampus, midfrontal cortex, and inferior temporal).

diagnosed AD, 46,828 proxy-ADD cases and 401,577 controls) and replication in 25,392 independent AD cases and 276,086 controls implicated rs5848 as a genome-wide significant locus for AD.<sup>44</sup>

We evaluated the association of rs5848 with neuropathological, behavioral, and cognition traits (Table 3, Table S4) using unadjusted

and adjusted models for age, sex, AD diagnosis, and APOE ε4 dosage. The rs5848 SNP was modestly associated with presence of hippocampal sclerosis (cor = 0.09, P = 3.07e-03) and TDP-43 pathology (cor = 0.082, P = 0.01), adjusting for age, sex, and AD diagnosis. The association was significant after adjusting for APOE ε4 status. Within

**TABLE 4** Frequency of Hippocampal Sclerosis (HS) and TDP-43 pathology in *GRN* rs5848 carriers in ROSMAP

rs5848 T alleles	Healthy at Autopsy (No AD)		Neuropathologically Confirmed AD	
	HS Absent	HS Present	HS Absent	HS Present
0	0.50	0.27	0.48	0.43
1	0.41	0.46	0.44	0.42
2	0.09	0.27	0.08	0.14

  

rs5848 T alleles	Healthy at Autopsy (No AD)		Neuropathologically Confirmed AD	
	TDP-43 Absent	TDP-43 Present	TDP-43 Absent	TDP-43 Present
0	0.53	0.43	0.50	0.47
1	0.40	0.43	0.43	0.43
2	0.07	0.14	0.07	0.10

Abbreviation: ROSMAP, Religious Orders Study and Memory and Aging Project.

<sup>a</sup>Hippocampal Sclerosis (% of individuals with rs5848 within each category (AD and HS absent, AD and HS present, Healthy and HS absent, healthy and HS present),  $P = 0.016$  in 6-df chi-square test. Raw numbers are shown in (Table S5).

<sup>b</sup>TDP-43 Pathology (% of individuals with rs5848 within each category (AD and TDP-43 pathology absent, AD and TDP-43 pathology present, Healthy and TDP-43 pathology absent, healthy and TDP-43 pathology present),  $P = 0.16$  in 6-df chi-square test. Number of individuals in each cell is given in Table S5.

homozygous rs5848 carriers with pathological AD, 17.4% had concomitant hippocampal sclerosis and 68% exhibited some TDP-43 pathology (9.7% and 58% for hippocampal sclerosis and TDP-43, respectively, amongst rs5848 non-carriers or heterozygotes; Table 4, Table S5).

### 3.4 | *GRN* mutations in the autopsied cohort of NACC WES

We used WES data from the ADSP<sup>29</sup> and neuropathological measures obtained from NACC to evaluate the frequency of *GRN* variants. Overall, we identified 30 putatively deleterious *GRN* variants in the NACC cohort. Among 3,252 individuals, for whom autopsy information was available, 31 (1%) individuals carried a *GRN* mutation (MAF = 0.0047) which is lower compared to the ROSMAP cohort. The low frequency here may be partially explained by the intersection of capture regions of the various exome kits used in the ADSP,<sup>29</sup> which could reduce the reliability of regions called within the gene. We evaluated the frequency of FTLT-tau using the variables specified in the NACC neuropathological dataset. Three out of fifteen individuals (20%) who were patients with postmortem AD and carrying a *GRN* mutation showed criteria of FTLT (as described below). In patients with clinical AD who did not carry a *GRN* mutation, presence of FTLT neuropathological features was observed at 5.5% ( $P$ -value = 0.063).

The three patient examples reveal the variation in *GRN* related neurodegeneration. Patient A, with clinical AD, carried a *GRN* mutation and had the pathological hallmarks of AD including Braak Stage = 5, Consortium to Establish a Registry for Alzheimer's Disease (CERAD) C score of 2 (moderate neuritic plaques), and NIA-AA ADNC score of 3 (high and frequent diffuse plaques). The patient had little tau pathology (FTLT-tau) but TDP-43 immunoreactive inclusions in the amygdala were observed. Patient B also had both clinical and pathological AD (Braak Stage = 5, CERAD C score = 3, and NIA-AA ADNC score = 3).

Concomitantly, TDP-43 immunoreactive inclusions were widespread in the amygdala, hippocampus, inferior temporal cortex, and neocortex. Interestingly both patients carried the *GRN* p.Arg433Trp mutation. Patient C (p.Val8Met mutation) was diagnosed as clinical AD but did not have the pathological hallmarks of AD (Braak Stage = 0, CERAD C score = 0 [no neuritic plaques and no diffuse plaques]) at autopsy. The patient had FTLT with parkinsonism, tau-positive, or argyrophilic inclusions and tauopathy but without ubiquitin-positive (tau-negative) inclusions.

### 3.5 | *GRN* mutations in families

To investigate the clinical characteristics of AD in pathogenic *GRN* carriers, we compared the frequency of behavioral and other psychiatric manifestations in EFIGA families between carrier and non-carrier status in living patients with AD. The presence of FTLT-like behavioral symptoms was assessed on the ten-point MFS.<sup>40</sup> The frequency of individuals with at least one behavioral symptom consistent with FTLT was compared between pathogenic *GRN* carriers and non-carriers. Medical record reviews were conducted in all *GRN* carriers and a similar number of randomly selected non-carriers to assess behavioral, mood, and psychosis-like symptoms.

In clinically diagnosed AD, there was no difference (Table S1) in the presence of FTLT-like symptoms on the MFS scale between carriers and non-carriers of pathogenic *GRN* variants (9% in carriers vs 11% in non-carriers) or between carriers and non-carriers of the common rs5848 SNP (Tables S1, S2). Interestingly, within unaffected family members, carriers of *GRN* variants and the common rs5848 SNP were more likely to have behavioral symptoms, assessed using the MFS (5.4% in carriers vs 1.3% in non-carriers,  $P = 0.03$ ). We found that 3.7% of the individuals carrying a *GRN* mutation also displayed parkinsonism while it was absent in non-carriers. Four patients in one family with clinical AD (Figure S4) and with a *GRN* splice variant (rs72824736)

had learning disabilities and one patient carrying another splice variant (rs112873166) had progressive aphasia. These observations were not present among non-carriers.

## 4 | DISCUSSION

GRN mutations explain up to 20% of familial and 5% of sporadic FTLD but lead to a variety of clinical presentations, predominantly presenting as behavioral variant FTLD or progressive aphasia. Less frequently, variants in GRN are found in clinical AD with or without parkinsonism. Among patients with clinical AD and not carrying mutations in PSEN1, PSEN2, and APP, 6.3% carried putatively pathogenic GRN mutations.<sup>45</sup> The authors recommend re-examination of clinical AD patients, particularly those who were diagnosed prior to identification of causal FTLD genes including GRN.

In this report, we systematically evaluated the frequency of putatively pathogenic GRN mutations in two large autopsy cohorts and one clinical cohort, and further examined the presence of concomitant tauopathy or other FTLD-like neuropathological or clinical presentations among patients with AD. In addition, we also examined the frequency of FTLD-like symptoms in patients with AD carrying rs5848, the strongest variant linked to FTLD-TDP43 pathology.

We found a higher than expected frequency of pathogenic GRN mutations among autopsied and clinically diagnosed AD compared to publicly available exome and genome datasets (gnomAD).<sup>46</sup> In the ROSMAP cohort, we found an association between rs5848 and hippocampal sclerosis and TDP-43 pathology. It has been previously reported that up to 25% to 50% of patients with AD have been found to have TDP-43 pathology at autopsy,<sup>47</sup> especially those with hippocampal sclerosis. However, in carriers of the rs5848 SNP, we found that 60% of pathologically confirmed AD patients exhibited TDP-43 pathology, and it increased to 67% if they were homozygous for the variant (Table S5). Interestingly, 95% (41 out of 43) rs5848-positive, AD patients presenting with hippocampal sclerosis also had TDP-43 pathology. Among the collection of Hispanic families, we found learning disabilities and aphasia concomitant with clinical AD in GRN carriers, but this was absent in non-carriers. GRN variants are present in ~16% primary progressive aphasia, 7% of behavioral-FTLD, and ~5% of AD with learning disabilities<sup>48,49</sup> suggesting that increased language and behavioral deficits in the presence of GRN variants in clinically diagnosed AD.

There are some limitations of this study including the diverse ascertainment and neuropathological characterizations across the autopsy cohorts. The in-silico pathogenic classification of GRN variants requires additional validation. Patients with mixed AD and FTLD presentations that carry GRN mutations with incomplete penetrance or mutations in other genes such as MAPT and C9orf72 would be missed in this analysis.

Progranulin levels in CSF are associated with the progression of early and late onset, clinically diagnosed AD.<sup>10</sup> In addition, progranulin levels are also associated with cortical thinning on brain MRI<sup>50</sup> and AD neuropathology.<sup>51</sup> Future studies should attempt to relate CSF progranulin levels, GRN variants, neurofibrillary tangle pathology, and Braak stage.

Taken together, the data presented here indicate that both rare and common GRN variants are associated with specific neuropathological findings in AD that are also present in FTLD. Postmortem data reveal that among neuropathologically diagnosed AD with GRN mutations, Braak stage and tau pathology exceed what is normally present in AD. Interestingly, GRN variants in AD were not accompanied by the typical behavioral manifestations occurring in FTLD. While GRN variants are strongly associated with FTLD, this report validates the numerous studies indicating that they can also be present in AD, but are not causal. As suggested earlier, it is possible that progranulin impacts AD, FTLD, and other neurodegenerative disease putatively by its effect on lysosomal storage in neurons and microglia.<sup>5</sup> Progranulin mutations may also explain concomitant tauopathies or other manifestations in AD neuropathology.

## ACKNOWLEDGMENTS

ROSMAP was supported by NIA grants P30AG10161, R01AG15819, R01AG17917, R01AG42210, U01AG46152, and U01AG61356. ROSMAP data can be requested at <https://www.radc.rush.edu>.

The EFIGA study is supported by NIA grants R56AG063908, R01AG067501 and RF1AG015473, and the whole genome sequencing in the families was supported by UM1HG008901. We acknowledge the services of CEDIMAT for collaborating with sample collection and processing in the EFIGA cohort.

The Alzheimer's Disease Sequencing Project (ADSP) is comprised of two Alzheimer's Disease (AD) genetics consortia and three National Human Genome Research Institute (NHGRI) funded Large Scale Sequencing and Analysis Centers (LSAC). The two AD genetics consortia are the Alzheimer's Disease Genetics Consortium (ADGC) funded by NIA (U01 AG032984), and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) funded by NIA (R01 AG033193), the National Heart, Lung, and Blood Institute (NHLBI), other National Institute of Health (NIH) institutes and other foreign governmental and non-governmental organizations. The Discovery Phase analysis of sequence data is supported through UF1AG047133 (to Drs. Farrer, Haines, Mayeux, Pericak-Vance, and Schellenberg); U01AG049505 to Dr. Seshadri; U01AG049506 to Dr. Boerwinkle; U01AG049507 to Dr. Wijsman; and U01AG049508 to Dr. Goate, and the Discovery Extension Phase analysis is supported through U01AG052411 to Dr. Goate, U01AG052410 to Dr. Pericak-Vance and U01 AG052409 to Drs. Seshadri and Fornage. Data generation and harmonization in the Follow-up Phases is supported by U54AG052427 to Drs. Schellenberg and Wang. The ADGC cohorts include: Adult Changes in Thought (ACT supported by NIA grant U01AG006781 to Drs. Larson and Crane), the Alzheimer's Disease Centers (ADC), the Chicago Health and Aging Project (CHAP), the Memory and Aging Project (MAP), Mayo Clinic (MAYO), Mayo Parkinson's Disease controls, University of Miami, the Multi-Institutional Research in Alzheimer's Genetic Epidemiology Study (MIRAGE), the National Cell Repository for Alzheimer's Disease (NCRAD), the National Institute on Aging Late Onset Alzheimer's Disease Family Study (NIA-LOAD), the Religious Orders Study (ROS), the Texas Alzheimer's Research and Care Consortium (TARC), Vanderbilt University/Case Western Reserve University (VAN/CWRU), the Washington

Heights-Inwood Columbia Aging Project (WHICAP supported by NIA grant RF1AG054023 to Dr. Mayeux) and the Washington University Sequencing Project (WUSP), the Columbia University Hispanic-Estudio Familiar de Influenza Genetica de Alzheimer (EFIGA supported by NIA grant RF1AG015473 to Dr. Mayeux), the University of Toronto (UT), and Genetic Differences (GD). Analysis of ADGC cohorts is supported by NIA grants R01AG048927 and RF1AG057519 to Dr. Farrer. Efforts of ADGC investigators were also supported by grants from the NIA (R03AG054936) and National Library of Medicine (R01LM012535). The CHARGE cohorts are supported in part by National Heart, Lung, and Blood Institute (NHLBI) infrastructure grant R01HL105756 (Psaty), RC2HL102419 (Boerwinkle), and the neurology working group is supported by the National Institute on Aging (NIA) R01 grant AG033193. The CHARGE cohorts participating in the ADSP include the following: Austrian Stroke Prevention Study (ASPS), ASPS-Family study, and the Prospective Dementia Registry-Austria (ASPS/PRODEM-Aus), the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Erasmus Rucphen Family Study (ERF), the Framingham Heart Study (FHS), and the Rotterdam Study (RS). ASPS is funded by the Austrian Science Fond (FWF) grant number P20545-P05 and P13180 and the Medical University of Graz. The ASPS-Fam is funded by the Austrian Science Fund (FWF) project I904, the EU Joint Programme - Neurodegenerative Disease Research (JPND) in frame of the BRIDGET project (Austria, Ministry of Science) and the Medical University of Graz and the Steiermärkische Krankenanstalten Gesellschaft. PRODEM-Austria is supported by the Austrian Research Promotion agency (FFG) (Project No. 827462) and by the Austrian National Bank (Anniversary Fund, project 15435. ARIC research is carried out as a collaborative study supported by NHLBI contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). Neurocognitive data in ARIC are collected by U01 2U01HL096812, 2U01HL096814, 2U01HL096899, 2U01HL096902, 2U01HL096917 from the NIH (NHLBI, NINDS, NIA and NIDCD), and with previous brain MRI examinations funded by R01-HL70825 from the NHLBI. CHS research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the NHLBI with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629, R01AG15928, and R01AG20098 from the NIA. FHS research is supported by NHLBI contracts N01-HC-25195 and HHSN268201500001. This study was also supported by additional grants from the NIA (R01s AG054076, AG049607 and AG033040 and NINDS (R01NS017950). The ERF study as a part of EUROSPAN (European Special Populations Research Network) was supported by European Commission FP6 STRP grant number 018947 (LSHG-CT-2006-01947) and also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the program "Quality of Life and Management of the Living Resources" of 5th Framework Programme

(no. QLG2-CT-2002-01254). High-throughput analysis of the ERF data was supported by a joint grant from the Netherlands Organization for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the municipality of Rotterdam. Genetic data sets are also supported by the Netherlands Organization of Scientific Research NWO Investments (175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), and the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA), project 050-060-810. All studies are grateful to their participants, faculty and staff. The content of these manuscripts is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the U.S. Department of Health and Human Services. The ADES-FR study was funded by grants from the Clinical Research Hospital Program from the French Ministry of Health (GMAJ, PHRC, 2008/067), the CNR-MAJ, the JPND PERADES, the GENMED labex (LABEX GENMED ANR-10-LABX-0013), and the FP7 AgedBrainSysBio. Whole exome sequencing in the 3C-Dijon study was funded by the Fondation Leducq. This work was supported by the France Génomique National infrastructure, funded as part of the Investissements d'Avenir program managed by the Agence Nationale pour la Recherche (ANR-10-INBS-09), the Centre National de Recherche en Génomique Humaine, the National Foundation for Alzheimer's disease and related disorders, the Institut Pasteur de Lille, Inserm, the Lille Métropole Communauté Urbaine council, and the French government's LABEX (laboratory of excellence program investment for the future) DISTALZ grant (Development of Innovative Strategies for a Transdisciplinary approach to Alzheimer's disease). The 3C Study supports are listed on the Study Website ([www.three-city-study.com](http://www.three-city-study.com)). The FinnAD Study at the University of Tampere was supported by The Academy of Finland: grants 286284 (T.L), Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation for Cardiovascular Research; Finnish Cultural Foundation; Tampere Tuberculosis Foundation; Yrjö Jahnsson Foundation; Signe and Ane Gyllenberg Foundation; and Diabetes Research Foundation of Finnish Diabetes Association. The FinnAD Study at the University of Eastern Finland was supported by the Academy of Finland grant 307866, the Sigrid Jusélius Foundation, and the Strategic Neuroscience Funding of the University of Eastern Finland. The three LSACs are: the Human Genome Sequencing Center at the Baylor College of Medicine (U54 HG003273), the Broad Institute Genome Center (U54HG003067), and the Washington University Genome Institute (U54HG003079).

Biological samples and associated phenotypic data used in primary data analyses were stored at Study Investigator institutions, and at the National Cell Repository for Alzheimer's Disease (NCRAD,



U24AG021886) at Indiana University funded by NIA. Associated Phenotypic Data used in primary and secondary data analyses were provided by Study Investigators, the NIA funded Alzheimer's Disease Centers (ADCs), and the National Alzheimer's Coordinating Center (NACC, U01AG016976) and the National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS, U24AG041689) at the University of Pennsylvania, funded by NIA, and at the Database for Genotypes and Phenotypes (dbGaP) funded by NIH. This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Library of Medicine. Contributors to the Genetic Analysis Data included Study Investigators on projects that were individually funded by NIA, and other NIH institutes, and by private U.S. organizations, or foreign governmental or nongovernmental organizations.

### CONFLICT OF INTEREST

Each co-author's conflict of interest is listed below.

D.R.D, A.L.P, M.M., D.R., I.Z.J, Y.Y.L and R.M. do not have any conflicts of interest.

B.N.V is a cancer bioinformatics consultant for Kodikaz Therapeutics

R.A.L. holds a position in the Nasry Michelen Foundation and was compensated for work-related travel through Columbia University. He has received funding from the National Institute of Health (NIH)

E.M. and M.P.V have received funding from the NIH

W.B was a consultant with Circumvent Pharmaceuticals. He has received funding through NIH, DiaComp Pilot and Feasibility Program, Foundation for Food and Agriculture

L.F. is a consultant with Gerson Lehrman Group

J.H.L has received consulting fees from the University of Miami. He holds grants from NIH

L.S.W received honoraria from Taiwan Neurological Society, National Chengchi University, East Tennessee State University, National Taiwan University. He is a member of the Emerging Information and Technology Conference (unpaid) and holds grants from the National Institute on Aging at NIH

Y.Y.L received grants from the NIH

G.S received honoraria from Brightfocus Foundation, Cure PSP, Seattle Veterans Affairs, and London OPDC. He has received grants from NIH

W.A.K. received payments/honoraria for grant reviews or advisory consultation from ADRCs in Boston University, University of California Irvine, University of Southern California, University of Kansas, Icahn School of Medicine, Texas Alzheimer's Research and Care Consortium (TARCC), Australia Brain Research and Brain Canada. He received consulting fees from Biogen and GSK and speaking fee from Peking University. He has grant funding from the NIH

P.D.J is a consultant with Biogen, Roche, Puretech and G.S.K. He has received grant funding from NIH, NMSS, FNIH, Roche, Biogen and Puretech

D.A.B. is a part of AbbVie's data monitoring board, a consultant with Takeda Inc, Origent Inc and SBIR. He consults with Vigorous Minds (unpaid). He has received NIH funding is a member of professional soci-

eties including the National Academy of Sciences and has given grand rounds talks.

J.S. received consulting fees from Alnylam, AVID radiopharmaceuticals, National Hockey League, and Framingham Study. She has NIH funding and has received honoraria for lectures at Rush University

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Vardarajan BN, Reyes-Dumeyer D, Piriz AL, et al. Progranulin mutations in clinical and neuropathological Alzheimer's disease. *Alzheimer's Dement*. 2022;1-10. <https://doi.org/10.1002/alz.12567>